CHAPTER 6

PULMONARY DIFFUSION ABNORMALITIES IN RELATION TO CYTOMEGALOVIRUS ANTIGENEMIA AND CYTOMEGALIC ENDOTHELIAL CELLS IN BLOOD

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ABSTRACT

The pathophysiology of CMV infection may involve many different organs including the lungs. In this study we investigated CMV antigenemia levels and cytomegalic endothelial cells (CEC) in blood in relation to the pulmonary diffusion capacity.

Patients with high CMV antigenemia (≥ 100 $pp65^+$ PMNs/50.000) (n = 8) showed a more extensive decrease in the membrane factor (Dm) than patients with lower levels of CMV antigenemia (n = 7). The decline of the diffusion capacity of the alveolar capillary membrane (KCOc) and of the pulmonary capillary volume (Vcap) was the same in both groups. Four out of nine patients had CEC in the range of 0.22 CEC/ml to 30.26 CEC/ml. All the CMV patients showed a decreased KCOc together with a decrease of Dm and Vcap but no difference was observed between patients with and without CEC. We conclude that a higher viral load is associated with a more extensive decrease in the membrane factor and therefore with more subclinical pneumonitis. No relation was observed between CEC and pulmonary dysfunction. Therefore, we postulate that CEC levels are related indirectly to subclinical pneumonitis mediated via the viral load.

6.1 INTRODUCTION

Although cytomegalovirus (CMV) infection is one of the most frequent infectious complications after organ transplantation, the pathophysiology of CMV disease remains speculative. Introduction of the cytomegalovirus pp65 antigenemia assay enabled early and rapid diagnosis of CMV-viremia prior to symptoms in transplant patients [1]. After kidney transplantation, cytomegalovirus infection most often causes a flu-like syndrome with fever, arthralgia, leukocytopenia, thrombocytopenia and elevated liver enzymes in symptomatic patients. The number of pp65 positive polymorphonuclear cells per 50.000 leukocytes is related to clinically symptomatic CMV disease [2]. The majority of patients with positive CMV antigenemia remain asymptomatic because of preemptive treatment with ganciclovir in case of institution of antirejection therapy. However, those still asymptomatic patients may have subclinical manifestations of CMV. We demonstrated, for example, increased intestinal mucosal permeability for disaccharides like lactulose in kidney transplant recipients with CMV infection [3]. Cytomegalovirus causes subclinical pulmonary dysfunction in kidney transplant recipients with or without CMV-associated symptoms [4, 5]. Whether the disturbed pulmonary diffusion or damage of intestinal integrity precede or coincidence with clinical pneumonitis or colitis is unknown. An important pathophysiological role was suggested for cytomegalic endothelial cells (CECs). CECs can be detected in peripheral blood in half of the patients with high pp65-antigenemia levels [5] and are related to high antigenemia levels [7, 8].

In a previous study [4] we found a decrease in pulmonary diffusion during CMV infection due to a combination of a lower membrane factor (Dm) and a lower pulmonary capillary volume (Vcap). We concluded that a local inflammatory process is the most likely explanation for the decrease in pulmonary diffusion as opposed to plugging of circulating cytomegalic endothelial cells (diameter 35-45 μm) in the pulmonary capillaries. In our opinion endothelitis and dissemination of the virus by circulating cytomegalic endothelial cells is important in the pathophysiology of CMV disease. In a recent study we demonstrate that the incidence of CEC was associated with CMV-related clinical symptoms [6]. In this study we investigated the relation between antigenemia levels, CEC in blood and the decline in pulmonary diffusion in 15 kidney transplant patients.

6.2 PATIENTS AND METHODS

Fifteen kidney transplant patients who developed CMV infection were included in this study. All patients had received a cadaveric transplant. None of the patients had a history of pulmonary disease and all had a normal physical examination and chest X-ray during the study period. Patients with postoperative cardiopulmonary complications, such as myocardial ischemia or infarction, pulmonary embolism, or bronchopneumonia, were excluded from the study. Initial immunosuppression consisted of cyclosporin A and low dose prednisolone. One patient received an induction course of OKT3. All patients gave their informed consent before participating in the study.

Pulmonary function was determined in all patients at approximately 15 days after transplantation (baseline value) and at least twice during CMV infection (median number of measurements 5, range 2 - 15). The transfer factor (diffusion capacity) for CO (TlCO) and its components, the diffusion capacity of the alveolar-capillary membrane (Dm) and the volume of blood in the pulmonary capillaries (Vcap), were determined from triplicate measurements of TlCOc at high (88%) and low (19,2%) concentrations of inspired oxygen. The single breath technique of Krogh, as modified by Cotes was used [9]. Carbon monoxide was measured with an infrared spectrophotometer and helium using a thermal conductivity method (ML-Masterlab-transfer; Jaeger, Germany). The TlCO values were corrected for hemoglobin concentrations (TlCOc), according to Cotes [9]. Corrected, specific diffusion capacity (KCOc) was calculated by dividing TlCOc by the alveolar volume. The Dm and the Vcap were derived from the equation of Roughton and Forster [10]:

$$1/TlCO = 1/Dm + 1/(\theta[Hb].Vcap$$

In this reaction, θ is the reaction rate of CO with hemoglobin (Hb) at the average normal Hb concentration (9 mmol/l). [Hb] is the hemoglobin concentration as a fraction of the average normal Hb concentration. Values are expressed as percentages of those predicted, the predicted values being taken from Cotes et al. [11] and Quanjer et al. [12].

Patients were monitored for CMV pp65-antigenemia twice a week. This test was performed according the procedure recently reviewed for standardization [1].

No CMV prophylaxis such as ganciclovir, acyclovir or hyperimmune gammaglobulin was given. Eight patients received ganciclovir because of clinical symptoms associated with rising CMV antigenemia values or preemptive because of antirejection treatment. CEC in blood was studied at approximately 15 days after transplanta-

tion (i.e. before infection) and weekly during CMV infection. This was continued until the CMV antigenemia was negative (n = 4) or less than 5/50.000 cells (n = 5).

CEC in blood were analyzed as has been described recently [6]. In brief, mononuclear cells (MNC) were isolated by density centrifugation using Lymfoprep (Nycomed Pharma AS, Oslo, Norway). 1 x 10^5 MNC were cytocentrifuged on a slide. The cytospots were stained by indirect immunofluorescence with the following antibodies: C10/C11 directed against CMV pp65 and E1/1 2.3 directed to a 90 kDa cell surface antigen of endothelial cells [13]. Four cytospots were analyzed if the concentration of MNC/ml blood was 1.5 x 10^6 or less, otherwise 6 to 8 cytospots were analyzed. The number of analyzed slides represented a detection limit of 20 CEC/ml blood in 95% of all samples.

Statistical analysis was performed using Student's *t*-test for paired and unpaired samples.

Table 6.1 Differences in pulmonary CO diffusion before and during CMV infection.

A	+ CEC (n = 4)	- CEC (n = 5)	*P*
Δ KCOc (mean ± SD)	22.28 ± 22.00	23.00 ± 25.00	ns
Δ Vcap (mean ± SD)	17.03 ± 18.90	19.94 ± 18.00	ns
Δ Dm (mean ± SD)	28.70 ± 23.85	17.56 ± 13.80	ns

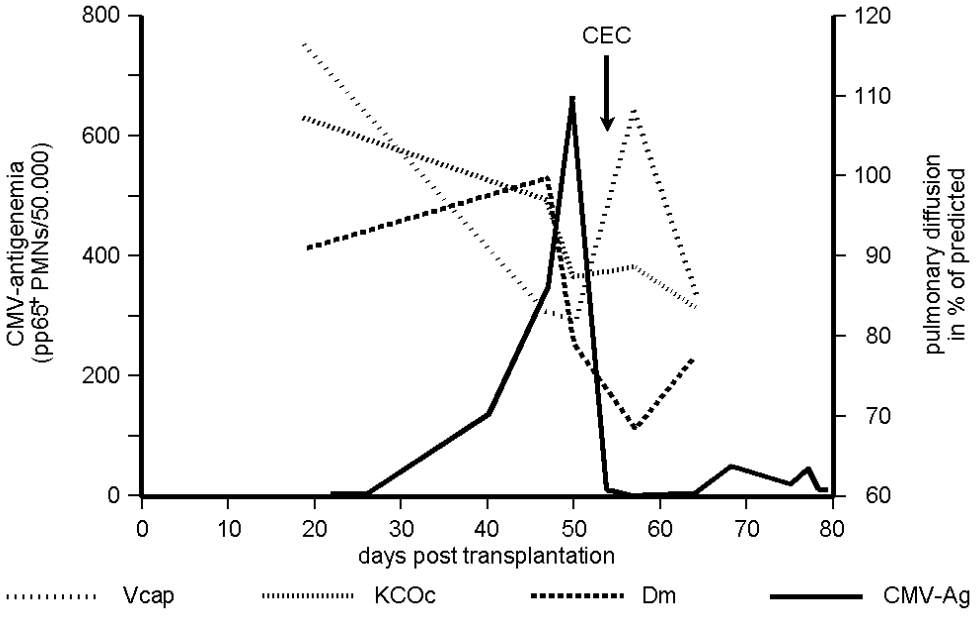
B	CMV-Ag ≥ 100* (n = 8)	CMV-Ag ≤ 100* (n = 7)	*P*
Δ KCOc (mean ± SD)	26.01 ± 6.12	25.00 ± 10.52	ns
Δ Vcap (mean ± SD)	21.01 ± 11.89	31.67 ± 21.25	ns
Δ Dm (mean ± SD)	29.48 ± 20.33	6.69 ± 12.26	$P < 0.05$

6.3 RESULTS

In this study, CO diffusion was determined in 15 patients (9 male / 6 female) with active CMV infection. The median age was 42 years (range 18 - 63 years). Eleven patients had a primary infection (positive donor organ, negative recipient) and the

remaining four patients had secondary infections (positive - positive combination). Nine patients had clinical symptoms such as fever, malaise, leukocytopenia, thrombocytopenia and elevated liver enzymes. Nine out of the fifteen patients were studied for CECs (table 6.1). CECs were observed in four out of these nine patients. In two patients CECs were observed once; in one patient twice, and in the last patient even six times during the course of CMV antigenemia. The CECs concentrations ranged from 0.22 CEC/ml to 30.26 CEC/ml (median 1.28 CEC/ml).

Figure 6.1 Typical course of CMV antigenemia (left Y-axis) and KCOc, Vcap and Dm (right Y-axis) in a kidney transplant recipient with CMV infection. The arrow indicates the occurrence of CEC.



The decrease in KCOc observed during CMV infection was similar for patients with and without CECs (22.28 ± 22.0 versus 23.0 ± 25.0, *P* = ns). The decrease in Vcap and Dm was also similar (17.03 +/□18.9 versus 19.94 ± 18.0, *P* = ns and 28.7 ± 23.85 versus 17.56 ± 3.8, *P* = ns, respectively) (table 6.1a).

The CO diffusion was analyzed in relation to the severity of infection. Patients with high CMV antigenemia (≥ 100 $pp65^+$PMNs/50.000) showed a more extensive decrease of Dm than patients with low or moderate CMV levels (<100 $pp65^+$ PMNs/50.000) (29.48 ± 20.33 versus 6.69 ± 12.26, $P < 0.05$). These differences were not observed in the KCOc and Vcap levels (26.01 ± 6.12 versus 25.0 ± 10.52,

P = ns and 21.01 ± 11.89 versus 31.67 ± 21.25, *P* = ns, respectively) (Table 6.1b). Patients with and without CMV associated clinical symptoms did not differ in decreases of KCOc, Vcap and Dm (KCOc: 25.57 ± 9.91 versus 25.0 ± 5.36, *P* = ns; Vcap: 28.34 ± 22.08 versus 22.45 ± 4.40, *P* = ns; Dm 17.99 ± 21.4 versus 20.12 ± 20.27, *P* = ns).

In figure 6.1 the course of KCOc, Vcap and Dm in a kidney transplant patient with CMV infection is illustrated. CEC were studied weekly and appeared four days after the maximal CMV antigenemia value. At that time the Dm and KCOc were decreased (32% and 8%) while the Vcap showed an increase of 22%.

6.4 DISCUSSION

During CMV infection, patients had a decreased pulmonary diffusion capacity that affected both Vcap and Dm. The balance between disturbances of the individual components was influenced by the severity of the infection, but we did not observe a specific influence of CEC in blood on either the Vcap or the Dm.

To clarify the contribution of CEC in blood, nine patients were studied for the occurrence of CEC. No evident differences in pulmonary diffusion capacity were observed between patients with and without CEC. Although the numbers were small, no tendency towards a diminished Vcap was observed. The numbers of CEC in patients varied between 0.22 CEC/ml to 30.26 CEC/ml, equivalent to 1.100 to 151.300 cytomegalic cells per 5 l of blood at that moment. Apparently, these numbers are too low to cause a measurable obstruction of the blood flow in the lungs, in addition to the decrease already observed in all CMV patients. Alternatively, the CECs might either be disrupted in the capillaries or be deformed and circulate normally, like genuine blood cells.

Because CECs are strongly related to high CMV antigenemia levels, we analyzed the severity of infection, as indicated by CMV antigenemia levels, in relation to CO diffusion as well. Patients were divided into groups with high CMV antigenemia levels ($\geq$ 100 $pp65^+$ PMNs/50.000) and moderate to low antigenemia levels ($<$ 100 $pp65^+$ PMNs/50.000). In the high CMV antigenemia group, the Dm decreased more than in the group with low CMV antigenemia, representing increased diffusion resistance from the alveolus to the capillaries. An inflammatory reaction with production of cytokines, fluid extravasation and cellular infiltrate could underlie these findings. The infiltrating cells can be composed of T cells, monocytes and macrophages [14]. Monocytes and macrophages are capable to produce nitric oxide (NO). NO has beside

immunomodulatory properties, strong vasoregulatory effects [15], which may (partially) compensate the decrease in Vcap.

In conclusion we found a significant decrease in Dm during more severe CMV infection. This indicates that the severity of subclinical pneumonitis is related to maximal antigenemia levels. We have not proven a relation between CECs and the decrease in Dm and Vcap. In the past, high viral loads expressed by high maximum antigenemia levels were related to CEC levels. We think that CEC levels are indirectly related to more extensive decreases in pulmonary diffusion, but because of low numbers of patients this could not be demonstrated.

6.5 REFERENCES

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